REMARKS

Rejection under 112 second paragraph

Claim 1 has been amended to address the rejection detailed in the Office Action. Withdrawal of the rejection is respectfully requested.

Rejections under 35 U.S.C. §103

The Office Action rejects claims 1-3 under 35 U.S.C. §103(a) as obvious over Nishikawa *et al.* (U.S. publ. no. 2004/0235160). Applicants submit that the Office Action fails to establish a *prima facie* case for obviousness. Further, there would be no likelihood of success of arriving at the present invention if Nishikawa *et al.* were modified as suggested in the Office Action.

First, Nishikawa *et al.* fail to teach isolation of mesenchymal stem cells (MSCs). As defined by claim 1, the present invention is directed to a method of isolating and culturing MSCs from cryopreserved umbilical cord blood (UCB), comprising the steps of thawing cryopreserved UCB and adding alpha-MEM media thereto, followed by centrifugation to harvest monocytes, isolation of $CD133^+$ cells from the obtained monocytes, and suspending the isolated cells into culture in alpha-MEM. The present invention is utilized to isolate a different cell type than Nishikawa *et al.* but the Office Action incorrectly states that "Nishikawa teaches methods for isolating and culturing mesenchymal cells..." (page 3, paragraph 2, Office Action). Hematopoietic stem cells

(HSCs), which are isolated by the process described in Nishikawa *et al.*, are defined as stem cells "having the multi-differentiating potential by self-renewing while they partly differentiate into various types of matured *blood cells* [emphasis added] via hematopoietic progenitor cells" (paragraph 004, Nishikawa *et al.*). The Office Action also inaccurately states that "...hematopoietic stem cells which [are] a source of mesenchymal cells..." (page 3, paragraph 3, Office Action). MSCs, which are the focus of the present invention, are defined as "primitive cells that are able to differentiate into bone, cartilage, adipose tissue, nerve, [and] muscle" (page 1, lines 15-16). On the other hand, HSCs are defined as "cell[s] having the ability to differentiate into all lineages of the *blood cells* [emphasis added] (multi-differentiation potential) and the self-renewability" (paragraph 0021, Nishikawa *et al.*). Thus, the claims and detailed description of Nishikawa *et al.* specifically and exclusively discuss HSCs, whereas the present claims and description specifically and exclusively discuss MSCs. The present invention encompasses different cell types, the MSCs, rather than the Nishikawa *et al.* publication, which encompasses the cell population of HSCs.

As described in the specification, the present invention provides a reproducible method of isolating and culturing MSCs from cryopreserved UCB (which is known as relatively lacking in such cells). Nonetheless, the presently claimed methods make it possible to secure primitive MSCs from UCB and improve the success rate of cell culture up to 90% (page 7, table 2). Applicants are not aware of any case reported in the scientific literature where MSCs are cultured from cryopreserved UCB using the conventional methods. Attached are three papers from the time the present invention was made demonstrating the absence of MSCs in UCB.

The scientific literature notes that isolating MSCs from UCBs has not been accomplished because UCB is generally a source of HSCs only: "it was possible to isolate MSCs from bone marrow but not from UCB" and "BM [bone marrow] contained mesenchymal stem cells that could easily be expanded and induced to differentiate for therapeutic use while the UCB adherent monolayer displayed the morphology and the characteristics of hematopoietic cells and not those of mesenchymal cells" (Mareschi *et al.*, Isolation of human mesenchymal stem cells: bone marrow versus umbilical cord blood, Haematologica 2001, 86: 1099-1100, quotes from p. 1099). Thus, prior to the present invention, no one has tried to obtain MSCs from UCB, which is not known to contain MSCs. Accordingly, a person skilled in the art at the time the invention was made would not have had a reasonable likelihood of success in isolating and culturing MSCs from cryopreserved UCB using the method described in the Nishikawa *et al.* publication.

The scientific literature also states that UCB is not a reliable source of MSCs: "adult BM [bone marrow] is a reliable source of functional cultured MSC, but CB [umbilical cord blood] and PBSC [peripheral blood stem cell collections] are not" (Wexler *et al.*, Adult bone marrow is a rich source of human mesenchymal 'stem' cells but umbilical cord and mobilized adult blood are not, British Journal of Haematology 2003, 121: 368-374, quote from p. 368). However, the present invention was able to overcome this limitation of obtaining MSCs from UCB by first enriching for monocytes that are $CD133^+$ and then culturing the isolates using enriched alpha-MEM media, neither of which is taught or suggested by the Nishikawa *et al.* publication. Further, the scientific literature clearly notes that "umbilical cord blood is a rich source of hematopoietic stem/progenitor cells and does not contain mesenchymal progenitors" except in minute

amounts since "MSCs circulate in the blood of preterm fetuses and *may* [emphasis added] be successfully isolated and expanded" (Romanov *et al.*, Searching for alternative sources of postnatal human mesenchymal stem cells: candidate MSC-like cells from umbilical cord, Stem Cells 2003, 21: 105-110, quote from p. 105). Thus, Nishikawa *et al.* was not isolating MSCs via the present method since an enrichment step involving $CD133^+$ cell isolation and replating is not taught or suggested by the Nishikawa *et al.* publication.

Accordingly, Nishikawa *et al.* fail to teach or suggest isolating MSCs and therefore cannot render the present claims obvious.

Finally, not only the source of cells but also the culturing conditions are different between Nishikawa *et al.* and the present invention. The Office Action erroneously states that "the instant [$CD133^+$] marker was a known marker of hematopoietic stem cells..." (page 3, paragraph 3, Office Action). Instead, $CD133^+$ is a marker of MSCs, which are obtained by "...isolating $CD133^+$ cells, and then culturing the cells in alpha-MEM including Stem Cell Factor, GM-CSF, G-CSF, IL-3, and IL-6, thereby making it possible to secure primitive mesenchymal stem cells and improve the success rate of cell culture" (page 3, line 27). As described in the specification of the present invention, it is difficult to isolate unadulterated monocytes from UCB since red blood cells are mixed in with these cells. For this reason and because of the near absence of MSCs in UCB, the present invention teaches selecting and culturing $CD133^+$ cells that encompass the MSC population. No disclosures in Nishikawa *et al.* publication would have led a person skilled in the art to isolate $CD133^+$ cells from UCB to obtain MSCs.

The Office Action also incorrectly states that "regarding the culture medium and the amount thereof, Nishikawa *et al.* te aches culturing mesenchymal cells in alpha-

MEM..." (page 3, paragraph 3, Office Action). Instead, the claims of Nishikawa *et al.* describe "a process for producing hematopoietic stem cells comprising the step of culturing hematopoietic stem cells in the presence of a gp130 stimulating factor, one or more cytokines, and stromal cells" (claim 1, Nishikawa *et al.*). The presently claimed method obtains MSCs, a different and broader stem cell type than HSCs, exclusively from cryopreserved UCB and does not utilize gp130 as its use would stimulate alternate differentiation pathways and thus alter the outcome of obtaining MSCs. Hence, Nishikawa *et al.* does not teach or suggest the results or methods of the present invention.

In summary, Nishikawa *et al.* isolate a different cell type than the present invention—namely, hematopoietic stem cells v. mesenchymal stem cells, respectively—with dissimilar possible ranges of cellular and tissue differentiation capacities. As such, the outcomes and possible therapies of Nishikawa *et al.* compared to the present invention are also very disparate, namely, treating blood and immune disorders (paragraph 0053, Nishikawa *et al.*) v. treating disorders of a variety of tissues from bone to nerve to muscle, among others (page 1, line 15, and page 7, line 25), respectively. Further, the perceived absence of MSCs from UCB at the time of the invention make it readily apparent that a person skilled in the art would not have been motivated to modify the invention of Nishikawa *et al.* to arrive at the present invention. The believed absence of MSCs also would have failed to suggest isolating and culturing $CD133^+$ cells from UCB. Finally, because of the absence of MSCs, one would not have expected such modification to be successful. As demonstrated and respectfully evidenced via the afore-presented arguments, the cited reference of Nishikawa *et al.* fails to teach or suggest the present invention.

Double Patenting

The double patenting concern is resolved by the currently filed terminal disclaimer form and the associated PTO 3.73(b) form. The rejection based on non-statutory obviousness is thus deemed moot.

CONCLUSIONS

Applicants maintain that all pending claims 1-5 are patentable and that, as such, the present application is in condition for allowance.

If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is hereby invited to telephone the undersigned at the number provided.

Applicants respectfully request that a Notice of Allowance of all pending claims 1-5 be timely issued in this case.

Respectfully submitted,

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